

WVU IACUC APPROVED STANDARD OPERATING PROCEDURE: OLAR ABSL-2 Use in Animals

Background:

Agents designated to be utilized at Biosafety Level 2 (BSL-2) are assigned by the Institutional Biosafety Committee (IBC) based on a risk assessment. ‘Risk Assessment’ is defined by the US CDC Biosafety in Microbiological and Biomedical Laboratories (BMBL) 6th edition as “a process used to identify the hazardous characteristics of a known infectious or potentially infectious agent or material, the activities that can result in a person’s exposure to an agent, the likelihood that such exposure will cause a Laboratory Acquired Infection, and the probable consequences of such an infection.”

Contact the Biosafety Officer (BSO) (phone: 304-581-1759) with any questions or concerns.

Definitions:

Animal Biosafety Level 2 (ABSL-2): Refers to animal use involving biological agents associated with human diseases designated as such by the Institutional Biosafety Committee (IBC).

Mixed Hazard: Refers to animals which received BOTH an ABSL-2 agent and a compound classified as a chemical hazard by the chemical safety officer.

Personal protective equipment (PPE): The appropriate amount and type of PPE required is determined by the IBC. PPE requirements for each room are posted on the animal housing room door.

Biosafety Cabinet (BSC): Primary engineering control that provides, user, sample, and environmental protection from biohazards.

Process to work with Biohazards in Animals:

ALL ANIMAL WORK INVOLVING HAZARDOUS AGENTS *MUST* TAKE PLACE IN APPROPRIATE DESIGNATED AREAS.

ANIMALS EXPOSED TO HAZARDS (BIOLOGICAL OR CHEMICAL) CANNOT BE HOUSED IN STANDARD HOUSING ROOMS DUE TO RISK OF EXPOSURE TO STAFF.

THESE ANIMALS *MUST* BE TRANSFERRED INTO DESIGNATED HOUSING LOCATIONS PRIOR TO HAZARD WORK COMMENCING.

1. An approved IBC and IACUC protocol describing the pathogens and the manner in which they will be used *must* be in place before work can begin. Personnel working with biohazards *must* also be added to the IBC protocol, for appropriate biosafety training to be assigned.
2. Principal Investigators working with biohazards and/or chemicals will be required to fill out an Animal Hazardous Use form prior to using those hazards. WVU Environmental Health & Safety (EHS) will send the form to the PI. The Animal Hazardous Use forms include information for OLAR staff on PPE requirements, bedding disposal, etc.

- a. Animal Hazardous Use forms are sent to the OLAR husbandry managers by EHS. These forms will be placed into the ABSL-2 housing room prior to the animals being exposed to the pathogen(s) are housed there.
3. Persons working in ABSL-2 areas **must** complete the following training:
 - a. Biosafety Level 2 training, first session in-person followed by training refresher on the SOLE website every 3 years.
 - b. Persons working with human tissues/blood or human derived cell lines **must** complete blood-borne pathogens training, first session in-person followed by training on SOLE annually.
 - c. In person animal training **must** be completed with an OLAR facility manager prior to using animals within the ABSL-2 space.
4. OLAR will grant access to the ABSL-2 suite after appropriate training is completed.
5. Once the laboratory is ready to utilize the hazardous agent, OLAR husbandry managers (OLARhusbandrysupervisors@hsc.wvu.edu) **must** be contacted at least 3 days ahead of time.
6. A Room-To-Room Animal Transfer Form (<https://hsc.wvu.edu/olar/forms/>) **must** be completed to transfer animals from standard housing room into the ABSL-2 suite to commence work with the hazards.
7. Once animals are transferred into the appropriate ABSL-2 room **by OLAR**, ABSL-2 work can begin.
8. ALL ABSL-2 work **must** be done in designated ABSL-2 housing or procedure rooms.

Rooms and areas designated as ABSL-2 will be subject to the following practices:

1. Biohazard signage will be posted on the doors of each ABSL-2/BSL-2 designated space and will list appropriate PPE and safety precautions.
2. Animal Hazardous Use forms are available in a binder in the animal housing room to advise on the hazard used and contains information for Occupational Medicine if an exposure occurs.
3. The cages in which infectious agents, including human cell lines, potentially exist **must** be labeled with a biohazard sticker attached to the cage card identifying the agent and a Hazardous Substance in Use card placed (see Appendix 1). If biohazard sticker affixed cages are brought to a lab, the lab door **must** have a biohazard sign with appropriate PPE listed, as well as emergency contact information.
4. If chemical hazards are used, a Hazardous Substance in Use card **must** be placed onto the cage as well. This card should indicate what hazard was administered and date of administration. This is now considered a mixed hazard cage and will be processed accordingly. If chemical hazards are used, an SDS sheet and Animal Hazardous Use form **must** be given to the OLAR husbandry

managers to place into the animal room. (see [WVU IACUC SOP: Hazardous Chemicals Used with Animals](#))

5. Workers will wear PPE for allergen exposure as per standard OLAR barrier housing procedures. N-95 respirators are not required, unless prescribed by Occupational Medicine or the IBC based on risk assessment. If an elevated risk assessment is established, appropriate signage will be posted on the room door.
6. All standard OLAR housing practices to enter or exit rodent holding or procedural rooms apply, including but not necessarily limited to: PPE practices, sharps handling, proper (BSC) use, appropriate waste disposal etc.
7. All PPE **must** be discarded in the biohazard waste before exiting the ABSL-2 designated room or suite.
8. All dirty cages
 - a. Biohazard cages (under ABSL-2 BSC)
 - i. All cages **must** be checked that animals and carcasses are removed prior to processing.
 - ii. Remove all disposable enrichment material from biohazard cage and dispose in biohazard bag. Re-usable enrichment items (huts) should be placed into a separate empty rodent cage for autoclaving.
 - iii. Cages that are not sealed using micro-isolator lids **must** be placed in clear autoclave bags and sealed with tape while those with micro-isolator tops can be left as is in the animal room.
 - iv. OLAR staff will remove these cages from the room and autoclave them prior to bedding disposal and cage washing.
 - v. **Biohazard cage drop is room: G241A**
 - vi. All biohazard cages are processed via autoclaving, followed by dumping.
 - b. Mixed hazard cages
 - i. Biohazards present in cage will be inactivated using a 10% bleach solution before disposing as chemical hazard waste. Bleach solution should be applied to the contents of cage so that it is saturated and remains wet for a minimum of 30 minutes.
 - ii. Cage cards should be marked to indicate the time and date the bleach spray was applied to the cage.
 - iii. Once disinfected, cages can be left in the animal housing room or placed on the chemical hazard cart in the dirty cage wash room: **V128**.
9. If a spill of biohazardous materials occurs, spill response forms listing the steps for cleaning up a spill are posted in all ABSL-2 rooms. Spill clean-up materials are available in all ABSL-2 rooms. The spill should be reported to the BSO.
10. Decontamination of surfaces and equipment **must** be done between cages using an appropriate high-level disinfectant provided by OLAR (e.g., PeroxiGard or similar accelerated hydrogen peroxide product).

11. Euthanasia and/or necropsy procedures **must** be done inside a certified BSC. Carcasses **must** be double bagged while in the hood, disinfected and deposited directly in the designated biohazard container in the cooler for disposal (**room V612 or V102**).
12. All materials used by the PI or lab staff (sample containers, instruments etc.) **must** be disinfected before removal from any ABSL-2 room.
13. HEPA filters **must** be used in the anesthesia circuit (typically before the scavenging system or canister) for gas anesthesia equipment, and a log kept of hours of use. After 24 hours of use (or for the period designated on the brand of filter unit used), HEPA filters must be replaced, and the older filter must be discarded into the designated BSL-2 waste bin in the room.
14. Sharps **must** be disposed of appropriately in a hard walled, labeled sharps disposal container. Any injury from a sharp object or infected animal **must** be treated immediately by scrubbing disinfectant that is supplied in each room. If necessary, the person will be referred to the Emergency Room. First aid kits with disinfectant are located in each OLAR hallway. An EHS incident report **must** be filed as soon as possible (forms at OLAR main office entry and on the EHS website). The BSO should be notified.
15. All ABSL-2 holding rooms housing immunodeficient (e. g., SCID, XID, Beige, RAG knockout, Nude, Non-obese diabetic SCID gamma chain knockout (NSG)) mice will have all bedding/cages/water bottles and other components that contact animals autoclaved before use.
16. Animals being transported from an ABSL-2 room are assumed to be infectious and **must** be placed in double-sealed containment such as in a cage with a lid in a secondary enclosure, such as a sealable plastic container or autoclave bag (only if short-term, so rodents do not risk suffocation or overheating). When leaving the vivarium, the cage **must** be obscured from public view using an opaque cover. Only the inner container needs have a biohazard sticker during transport. (see [WVU IACUC SOP: Hazardous Chemicals Used with Animals](#))
17. ABSL-2 use in large animals is done on a case-by-case basis in consultation with the BSO and IBC.

Testing Requirements for Human or Murine Origin Biologicals:

Human cells (cell lines, blood or primary tissue) or human origin reagents: Samples will be treated as if they are infectious, so no additional testing is required for human agents. However, testing is available and encouraged.

Rodent cells or rodent origin reagents: Testing of cell lines for murine pathogens is required. Frequency of testing and requirements for eliminating pathogens from cell lines will be addressed by OLAR. (see [WVU IACUC Policy: Pathogen Screening of Biological Material used in Rodents](#))

In house generated rodent origin tissue, reagents or blood: Procedures will be used based on the source and health status of the donor and recipient mice.

Appendix 1

Hazardous Substance in Use

(If using tumor cells, please use tumor score card)

Chemical / Biological

(Circle one below)

Mutagen / Teratogen / Carcinogen / Toxin

Substance Name: _____

Substance Quantity: _____

Substance Organism: _____

PI:	Phone #:
Contact:	Phone#:
Protocol #:	IBC#:
Cage ID:	Location:
Start Date:	End Date:

Route Given (circle one)

Water / Food / Injection / Gavage

Date	Amount Given	Initials

References

“Biosafety in Microbiological and Biomedical Laboratories (BMBL). US Centers for Disease Control.
<https://www.cdc.gov/labs/bmbl/index.html>